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PAR6C polarity protein expression pattern in male and female bovine embryos

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Despite the unique importance of blastomere polarization during preimplantation development, little is known about this phenomenon in bovine embryos. Polarization transforms symmetrical spherical blastomeres into highly polarized radial cells, and once established, polarity domains distinguish embryonic inner and outer cells. Cells exhibiting outer polarized domains cannot depolarize, and will contribute to trophectoderm lineage. Bovine embryos develop slower than mouse embryos, and at lower rates. Also, gender influences some characteristics in bovine embryos, including cell numbers and apoptosis. Therefore, the aim of this study was to characterize PAR6c, a polarity marker well established in mouse embryos, in bovine embryos, and investigate if its expression is similar in male and female counterparts. In experiment 1, bovine embryos (n= 52) were produced by IVF and embryo culture at standard conditions, in two replicates. Samples were collected at several stages of development (zygote, 2-, 4-, 8-, 16- morula and blastocyst), and immunofluorescence (IF) for PAR6c protein was performed. In experiment 2, male and female embryos (n=192) produced in three replicates using sexed semen from the same bull were cultured separately, and morphological embryo development assessment was performed daily from 24-144 h.p.i. Developmental rates were compared between groups at each evaluated time point using Chi-Square Test. In experiment 3, male and female embryos were collected (n=46) at critical stages (late 8; early 16; mid 16; late 16; morula) for PAR6c IF. Mouse embryos were used as positive controls for all PAR6c IF reactions. Negative control reactions were also performed. We observed PAR6c consistent apical expression from 16-cell embryos onwards; although about half embryos (9/17) exhibited very low expression already at 8-cell stage. Before 8-cell stage, no expression was detected. In experiment 2, we observed a difference in 8 to 16-cell embryo percentage between male and female embryos at 48h.p.i. (M=20%; F=34%*) and 72h.p.i (M=64%; F=43%*). In experiment 3, we observed that PAR6c expression and stage of compaction were similar in both groups, and were first evidently detected at early 16-cell stage. At late 8-cell stage, 60% male embryos and 80% female embryos had at least one blastomere expressing PAR6c, but at very low levels. We concluded that PAR6c asymmetric protein expression seems to be consistently established at 16-cell stage, one cell cycle after occurs in mouse embryos. This result suggests that cell fate decisions in bovine could also be started before blastocyst stage. We found that 48-72 h.p.i. is a critical stage for male and female embryos comparative analysis in our system. However, at this stage male and female embryos display similar pattern of PAR6c polarization and compaction, suggesting those events are not related to phenotypic differences previously reported between those groups.

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